Claims

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1. A structure which comprises a biological membrane adhered with a high resistance seal to a porous or perforated substrate for use in a high throughput screen wherein the biological membrane comprises an ion channel or transporter.

2. A structure according to claim 1 wherein the biological membrane comprises a contiguous layer of cells which is capable of adhering to a substrate with a high resistance seal wherein each cell forms a tight junction with adjacent cells and expresses an ion channel or transporter which is localised in the cell membrane.

3. A structure according to claim 1 or 2 which comprises cells having an ion channel or transporter which naturally resides in the cell membrane thereof or, it can be inserted by transfection with cDNA and/or cRNA encoding the ion channel or transporter.

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4. A structure according to any preceding claim which comprises a plurality of ions channels or transporters which are predominantly preselected ion channels or transporters of interest.

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5. A structure according to any preceding claim which comprises genetically engineered cells which have been engineered to predominantly express an ion channel or transporter.

6. A structure according to any preceding claim which comprises voltage gated ion channels.

7. A structure according to any one of claims 2 to 5 wherein the cells are selected from the group which comprises HEK-293 cells, genetically modified Chinese hamster ovary (CHO) cells, primary neuronal tissue such as hippocampus, dorsal root ganglia, superior cervical ganglia etc.; skeletal muscle; smooth muscle; cardiac muscle; immune cells; epithelia; endothelia.

8. A structure according to any preceding claim which comprises an ion channel having rapid activation and inactivation kinetics.

9. A structure according to any preceding claim having an ion channel which shows specificity for an ion selected from the group which comprises sodium, potassium, calcium, chloride.

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10. A structure according to any one of claims 2 to 9 wherein the contiguous layer of cells is capable of

adhering with a high resistance seal to a substrate selected from the group which comprises glass, plastics, rubber, polytetraflurotethylene (PTFE), PTFE/glass fabric and polyethylene terephthalate (PETP).

11. A structure according to any preceding claim which comprises a pseudo-epithelium wherein one face of a contiguous layer of cells is permeabilized thereby providing access to the interior of the cells.

12. A structure according to claim 11 which comprises a pseudo-epithelium wherein one face of the contiguous layer of cells is permeabilized by an antibiotic selected from the group which comprises amphotericin and nystatin; or detergent selected from the group which comprises digitonin and saponin; or physical disruption using a high voltage field; or by enzymatic digestion of a part of the membrane using an appropriate enzyme.

13. A structure according to any preceding claim wherein the substrate is perforated.

14. A structure according to any preceding claim which comprises a perforated coverslip.

15. A structure according to any preceding claim wherein the substrate has pores of diameters between 0.5μm and 10μm.

16. A structure according to claim 15 wherein the pores are of diameter between 1μm and 7μm.

17. A structure according to claim 15 or 16 wherein the pores are of diameter 1-2μm.

18. A structure according to any preceding claim which comprises a coverslip having a grid of pores.

19. A structure according to any preceding claim which comprises a perforated substrate which is manufactured of a material selected from the group which comprises glass, plastics, rubber, polytetraflurotethylene (PTFE), PTFE/glass fabric and polyethylene terephthalate (PETP).

20. A biological membrane for use in the structure according to any preceding claim.

21. A substrate for use in the structure according to any one of claims 1 to 19.

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22. A high throughput screen for detecting and assaying compounds with activity on voltage gated ions channels which comprises a structure according to any one of claims 1 to 19, a biological membrane according to any claim 20 or a substrate according to claim 21.

23. A high throughput screen according to claim 22 which comprises :

a plurality of chambers, each having a permeable peripheral surface providing a substrate for the biological membrane;

a plurality of wells each capable of receiving a chamber and a test compound in a physiological solution or non-physiological solution comprising dimethyl sulphoxide;

a plurality of reference electrodes, having electrical contact with each well;

a movable recording head carrying at least one recording electrode;

means for measuring electrical resistance or impedance between the recording and reference electrodes; wherein

electrical current may pass between the recording and reference electrodes through the permeable peripheral surface of each chamber only via ion channels or transporters in the biological membrane.

24. A high throughput screen according to claim 23 wherein the wells are provided by a multiwell plate.

25. A high throughput screen according to claim 22 which comprises an array of droplets on a porous substrate.

26. A high throughput screen according to any one of claims 22 to 25 which comprises a recording head having a single recording electrode capable of being moved to visit each chamber sequentially.

27. A high throughput screen according to any one of claims 22 to 25 which comprises a recording head having a plurality of recording electrodes arranged in a line.

28. A high throughput screen according to any one of claims 22 to 25 which comprises a recording head having a plurality of recording electrodes arranged in a matrix.

29. A method of manufacturing the structure of any one of claims 1 to 19 which comprises he steps of selecting a substrate, perforating it, introducing a biological

membrane to the substrate and sealing each pore with biological membrane.

30. A method according to claim 29 which comprises the steps of simultaneously perforating a substrate and sealing the pores with biological membrane.

31. A method of manufacturing a biological membrane according to claim 20 which comprises the steps of selecting a cell type, evaluating it for ability to form contiguous layers of cells with tight junctions and for low to negligible numbers of voltage gated ion channels, culturing the cells on a substrate and ensuring that a contiguous layer of cells is grown.

32. A method according to claim 31 which includes the step of permeabilizing one surface of the contiguous layer of cells thereby providing access to the interior of the cells.

33. A method according to claim 32 wherein the step of permeabilizing one surface of the contiguous layer of cells is carried out by the step of contacting the surface with an antibiotic selected from the group which comprises amphotericin and nystatin; or detergent selected from the group which comprises digitonin and saponin; or physical disruption using a high voltage

field; or by enzymatic digestion of a part of the cell membrane using an appropriate enzyme.

34. A method according to any one of claims 31 to 33 which comprises the steps of transfecting cells with cDNA or cRNA encoding an ion channel of interest and cloning cells expressing the ion channel of interest.

35. A method of manufacturing a substrate according to claim 21 which comprises the steps of shining a laser of preselected focal area, power or time of exposure at a coverslip to perforate it.

36. A method according to claim 35 which comprises the steps of adjusting the profile, taper or diameter of the pore with a laser.

37. A method according to claim 35 or 36 wherein the laser source is controlled by an automated stage under control of a computer and inverted optics microscope.

38. A method of manufacturing a substrate according to claim 21 which comprises a non-laser method selected from the group which comprises photo-etching, casting and physical piercing of the substrate.

39. A method of screening for the detection or assay of compounds with activity on ion channels or transporters which comprises the steps of placing a biological membrane which expresses ion channels or transporters of interest in contact with test compound in physiological solution or non-physiological solution comprising dimethyl sulphoxide and measuring the resistance or impedance of the biological membrane under the influence of test compound.

40. A method according to claim 39 wherein ion channel activity is monitored by trans-epithelial resistance measurements (TERM) across an intact cell layer.

41. A method according to claim 39 which comprises the step of permeabilizing a contiguous cell layer to provide access to the interior of the cells permitting intracellular voltage and current measurements to be made.

42. A method according to claim 41 wherein a contiguous cell layer is permeabilized by antibiotic selected from the group which comprises amphotericin and nystatin; or detergent selected from the group which comprises digitonin and saponin; or physical disruption using a high voltage field; or by enzymatic digestion of a part of the cell membrane using an appropriate enzyme

thereby permitting intracellular voltage or current measurements to made.

43. A method according to any one of claims 39 to 42 which includes the step of multiplexing up to 384 recording elements to a data acquisition system utilizing multiple voltage-clamp amplifiers.

44. A method according to any one of claims 39 to 43 which comprises the step of placing an array of droplets having ion channels or transporters of interest therein on a porous substrate and screening test compounds for activity on the ion channels or transporters.

45. A method according to any one of claims 39 to 43 which comprises the step of placing biological membrane having ion channels or transporters of interest and test compounds in physiological solution, or non-physiological solution comprising dimethyl sulphoxide, in a plurality of chambers and screening the test compounds for activity on the ion channels or transporters.

46. A method according to any of claims 21, 30 and 35 to 38 wherein, subsequent to pore formation, the substrate is exposed to localised heat and/or to electrical

plasma in order to impart an appropriate raised level of smoothness to the pore(s).